

The array was composed of oligonucleotide probes (25-30 mer) complementary to four virulence loci (intimin, Shiga-like toxins I and II, and hemolysin A). Target DNA was amplified from whole cells or from purified DNA via single or multiplexed polymerase chain reaction (PCR), and PCR products were hybridized to the array without further modification or purification. The array was 32-fold more sensitive than gel electrophoresis and capable of detecting amplification products from <1 cell equivalent of genomic DNA (1 fg). Immunomagnetic capture, PCR and a microarray were subsequently used to detect 55 CFU ml⁻¹ (E. coli O157:H7) from chicken rinsate without the aid of pre-enrichment. Four isolates of E. coli O157:H7 and one isolate of O91:H2, for which genotypic data were available, were unambiguously genotyped with this array. Glass-based microarrays are relatively simple to construct and provide a rapid and sensitive means to detect multiplexed PCR products; the system is amenable to automation.

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FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 10:59:19 ON 24 JUN 2007

L1 60 S ("FRET") AND (PATHOGEN) AND (FUNGAL OR (GRAM(W)NEGATIVE) OR (
L2 91 S ("FRET") AND (PATHOGEN) AND PROBE?
L3 425 S PATHOGEN AND PROBE? AND MULTIPLEX?
L4 148 S L3 AND (FUNGAL OR (GRAM(W)NEGATIVE OR GRAM(W) POSITIVE))
L5 232 S PATHOGEN? AND PROBE? AND (LIGHTCYCLER)
L6 52 DUP REM L1 (8 DUPLICATES REMOVED)
L7 79 DUP REM L2 (12 DUPLICATES REMOVED)
L8 326 DUP REM L3 (99 DUPLICATES REMOVED)
L9 137 DUP REM L4 (11 DUPLICATES REMOVED)
L10 184 DUP REM L5 (48 DUPLICATES REMOVED)
L11 24 S L1 AND PY<=2003
L12 24 S L6 AND PY<=2003
L13 47 S L7 AND PY<=2003
L14 170 S L8 AND PY<=2003
L15 91 S L9 AND PY<=2003
L16 117 S L10 AND PY<=2003

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L1      60 SEA ABB=ON  PLU=ON  ("FRET") AND (PATHOGEN) AND (FUNGAL OR
      (GRAM(W) NEGATIVE) OR (GRAM(W) POSITIVE))
L2      91 SEA ABB=ON  PLU=ON  ("FRET") AND (PATHOGEN) AND PROBE?
L3     425 SEA ABB=ON  PLU=ON  PATHOGEN AND PROBE? AND MULTIPLEX?
L4     148 SEA ABB=ON  PLU=ON  L3 AND (FUNGAL OR (GRAM(W) NEGATIVE OR
      GRAM(W) POSITIVE))
L5     232 SEA ABB=ON  PLU=ON  PATHOGEN? AND PROBE? AND (LIGHTCYCLER)
L6      52 DUP REM L1 (8 DUPLICATES REMOVED)
L7      79 DUP REM L2 (12 DUPLICATES REMOVED)
L8     326 DUP REM L3 (99 DUPLICATES REMOVED)
L9     137 DUP REM L4 (11 DUPLICATES REMOVED)
L10    184 DUP REM L5 (48 DUPLICATES REMOVED)
L11     24 SEA ABB=ON  PLU=ON  L1 AND PY<=2003
L12     24 SEA ABB=ON  PLU=ON  L6 AND PY<=2003
L13     47 SEA ABB=ON  PLU=ON  L7 AND PY<=2003
L14    170 SEA ABB=ON  PLU=ON  L8 AND PY<=2003
L15     91 SEA ABB=ON  PLU=ON  L9 AND PY<=2003
L16    117 SEA ABB=ON  PLU=ON  L10 AND PY<=2003
      D L12 TI 1-24
      D L13 TI 1-25
      D L13 TI 26-47
      D L15 TI 1-30
      D L15 TI 31-60
      D L15 TI 61-91
      D L12 IBIB ABS 1,3-5,10,13,16,21,23
      D L13 IBIB ABS 3,6,8,9,27,33,41,46
      D L15 IBIB ABS 5,8,18,22,40,51,65,74
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#12	Search pathogen and ("Lightcycler") Limits: Publication Date to 2003/12, only items with links to full text, only items with links to free full text	20:09:52	<u>4</u>
#11	Search pathogen and ("Lightcycler") and (fungal or gram (negative or positive)) Limits: Publication Date to 2003/12, only items with links to full text, only items with links to free full text	20:09:39	<u>0</u>
#6	Search pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive)) Limits: Publication Date to 2003/12, only items with links to full text, only items with links to free full text	19:26:00	<u>27</u>
#7	Search pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive)) Limits: Publication Date to 2004, only items with links to full text, only items with links to free full text	18:10:23	<u>35</u>
#5	Search pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive)) Limits: Publication Date to 2003/12	18:08:26	<u>68</u>
#4	Search pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive))	18:07:52	<u>122</u>

#3	Search pathogen and (hybridization or "Lightcycler")) and (fungal or gram (negative or positive))	18:07:38	<u>122</u>
#2	Search (pathogen and (hybridization or "Lightcycler")) and (fungal or gram (negative or positive))	18:07:25	<u>122</u>
#1	Search (pathogen and (hybridization or "Lightcycler")) and (fungal or gram (negative or positive))	18:06:51	<u>122</u>

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Quantitative test for bacterial pathogens

EJ Baron, WD Engel, G Haberhausen, P Kaspar, AM ... - 2006 - freepatentsonline.com
... for amplifying all types of **fungal** ribosomal 18S ... and subsequent analysis using **FRET**
hybridization probes ... is specific for said bacterial **pathogen** is indicative ...

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Method for the detection of pathogenic gram positive bacteria from the genera staphylococcus, ...

G Haberhausen, T Emrich, R Rossasu, G Jannes, DD ... - 2006 - freepatentsonline.com
... primers for amplifying all types of **fungal** ribosomal 18S ... of a **FRET** donor moiety and
a **FRET** acceptor moiety ... species or strain of the respective **pathogen** that has ...

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Method for the detection of pathogenic gram positive bacteria selected from the genera ...

GDRDB HABERHAUSEN, TDRDC EMRICH, R ROSSAU, GE ... - EP Patent 1,426,447, 2004 - freepatentsonline.com
... primers for amplifying all types of **fungal** ribosomal 18S ... of a **FRET** donor moiety and
a **FRET** acceptor moiety ... species or strain of the respective **pathogen** that has ...

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Innate immunity markers for rapid diagnosis of infectious diseases - all 2 versions »

PJ Dailey - 2003 - freepatentsonline.com
... resonance energy transfer assay "**FRET** assay"), reverse ... a "type of infectious **pathogen**"
includes determining ... eg, a bacterial, yeast, viral or **fungal** infection. ...

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TLR 4-dependent lipopolysaccharide signalling in epithelial cells is independent of extracellular ... - all 4 versions »

F Backhed, S Normark, A Richter-Dahlfors - Cellular Microbiology, 2002 - Blackwell Synergy
... TLRs), which discriminate potential **pathogens** from self. ... protease activities initiated
upon **fungal** infections, which ... et al., 2001) and **FRET** technology (Jiang ...

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Multiplex assay detection of pathogenic organisms - all 2 versions »

G Haberhausen, T Emrich, G Sagner, M Moczko, G ... - 2006 - freepatentsonline.com
... a **FRET** donor moiety and a **FRET** acceptor moiety ... of a predetermined group of pathogenic
fungi, said group ... is used to detect and identify **fungal pathogens** such as ...

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Innate immune responses to microbial poisons: Discovery and function of the toll-like receptors - all 5 versions »

B Beutler - Annual Review of Pharmacology and Toxicology, 2003 - phyto.annualreviews.org
... that Toll protects flies against **fungal** infection (68 ... resonance energy transfer
(**FRET**) analyses suggest ... small contribution to susceptibility for most **pathogens**. ...

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The Function of Mlo, A negative Regulator of Defence, is conserved in Monocot and Dicot plants

C Consonni - 2005 - kups.ub.uni-koeln.de
... **FRET** fluorescence resonance energy transfer ... structures essential for the survival
of the **pathogen**. ... glucans, chitins and proteins derived from **fungal** cell walls ...

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Charge Transfer Complexes by R. Foster, Academic Press, 1969), the laser energy can be tuned to the corresponding energy of the charge-transfer wavelength and, thus, a specific desorption off the solid support can be initiated. Those skilled in the art will recognize that several combinations can serve this purpose and that the donor functionality can be either on the solid support or coupled to the nucleic acid molecule to be detected or vice versa.

In yet another approach, a reversible L-L' linkage can be generated by homolytically forming relatively stable radicals. Under the influence of the laser pulse, desorption (as discussed above) as well as ionization will take place at the radical position. Those skilled in the art will recognize that other organic radicals can be selected and that, in relation to the dissociation energies needed to homolytically cleave the bond between them, a corresponding laser wavelength can be selected (see e.g., *Reactive Molecules* by C. Wenstrup, John Wiley & Sons, 1984).

When performing exonuclease sequencing using MALDI-TOF MS, a single stranded DNA molecule immobilized via its 5'-end to a solid support is unilaterally degraded with a 3'-processive exonuclease and the molecular weight of the degraded nucleotide is determined sequentially. Reverse Sanger sequencing reveals the nucleotide sequence of the immobilized DNA. By adding a selectively cleavable linker, not only can the mass of the free nucleotides be determined but also, upon removal of the nucleotides by washing, the mass of the remaining fragment can be detected by MALDI-TOF upon cleaving the DNA from the solid support. Using selectively cleavable linkers, such as the photocleavable and chemical cleavable linkers provided herein, this cleavage can be selected to occur during the ionization and volatilizing steps of MALDI-TOF. The same rationale applies for a 5' immobilized strand of a double stranded DNA that is degraded while in a duplex. Likewise, this also applies when using a 5'-processive exonuclease and the DNA is immobilized through the 3'-end to the solid support.

As noted, at least three version of immobilization are contemplated herein: 1) the target nucleic acid is amplified or obtained (the target sequence or surrounding DNA sequence must be known to make primers to amplify or isolated); 2) the primer nucleic acid is immobilized to the solid support and the target nucleic acid is hybridized thereto (this is for detecting the presence of or sequencing a target sequence in a sample); or 3) a double stranded DNA (amplified or isolated) is immobilized through linkage to one predetermined strand; the DNA is denatured to eliminate the duplex and then a high concentration of a complementary primer or DNA with identity upstream from the target site is added and a strand displacement occurs and the primer is hybridized to the immobilized strand.

In the embodiments where the primer nucleic acid is immobilized on the solid support and the target nucleic acid is hybridized thereto, the inclusion of the cleavable linker allows the primer DNA to be immobilized at the 5'-end so that free 3'-OH is available for nucleic acid synthesis (extension) and the sequence of the "hybridized" target DNA can be determined because the hybridized template can be removed by denaturation and the extended DNA products cleaved from the solid support for MALDI-TOF MS. Similarly for 3), the immobilized DNA strand can be elongated when hybridized to the template and cleaved from the support. Thus, Sanger sequencing and primer oligo base extension (PROBE), discussed below, extension reactions can be performed using an immobilized primer of a known, upstream DNA sequence complementary to an invariable

region of a target sequence. The nucleic acid from the person is obtained and the DNA sequence of a variable region (deletion, insertion, missense mutation that cause genetic predisposition or diseases, or the presence of viral/bacterial or fungal DNA) not only is detected, but the actual sequence and position of the mutation is also determined.

In other cases, the target DNA must be immobilized and the primer annealed. This requires amplifying a larger DNA based on known sequence and then sequencing the immobilized fragments (i.e., the extended fragments are hybridized but not immobilized to the support as described above). In these cases, it is not desirable to include a linker because the MALDI-TOF spectrum is of the hybridized DNA; it is not necessary to cleave the immobilized template.

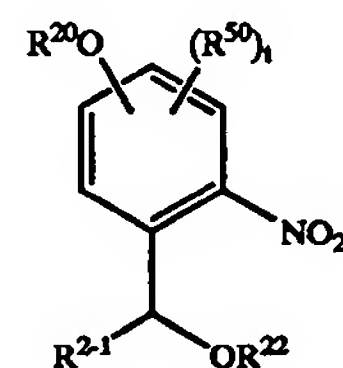
Any linker known to those of skill in the art for immobilizing nucleic acids to solid supports may be used herein to link the nucleic acid to a solid support. The preferred linkers herein are the selectively cleavable linkers, particularly those exemplified herein. Other linkers include, acid cleavable linkers, such as bismaleimideoxy propane, acid-labile trityl linkers.

Acid cleavable linkers, photocleavable and heat sensitive linkers may also be used, particularly where it may be necessary to cleave the targeted agent to permit it to be more readily accessible to reaction. Acid cleavable linkers include, but are not limited to, bismaleimideoxy propane; and adipic acid dihydrazide linkers (see, e.g., Fattom et al. (1992) *Infection & Immun.* 60:584-589) and acid labile transferring conjugates that contain a sufficient portion of transferrin to permit entry into the intracellular transferrin cycling pathway (see, e., Welhöner et al. (1991) *J. Biol. Chem.* 266:4309-4314).

Photocleavable Linkers

Photocleavable linkers are provided. In particular, photocleavable linkers as their phosphoramidite derivatives are provided for use in solid phase synthesis of oligonucleotides. The linkers contain o-nitrobenzyl moieties and phosphate linkages which allow for complete photolytic cleavage of the conjugates within minutes upon UV irradiation. The UV wavelengths used are selected that the irradiation will not damage the oligonucleotides and are preferably about 350-380 nm, more preferably 365 nm. The photocleavable linkers provided herein possess comparable coupling efficiency as compared to commonly used phosphoramidite monomers (see, Sinha et al. (1983) *Tetrahedron Lett.* 24:5843-5846; Sinha et al. (1984) *Nucleic Acids Res.* 12:4539-4557; Beaucage et al. (1993) *Tetrahedron* 49:6123-6194; and Matteucci et al. (1981) *J. Am. Chem. Soc.* 103:3185-3191).

In one embodiment, the photocleavable linkers have formula I:



where R^{20} is w-(4,4'-dimethoxytrityloxy)alkyl or w-hydroxyalkyl; R^{21} is selected from hydrogen, alkyl, aryl, alkoxy, carbonyl, aryloxy, carbonyl and carboxy; R^{22} is hydrogen or (dialkylamino)(w-cyanoalkoxy)P-; t is 0-3; and R^{50} is alkyl, alkoxy, aryl or aryloxy.